

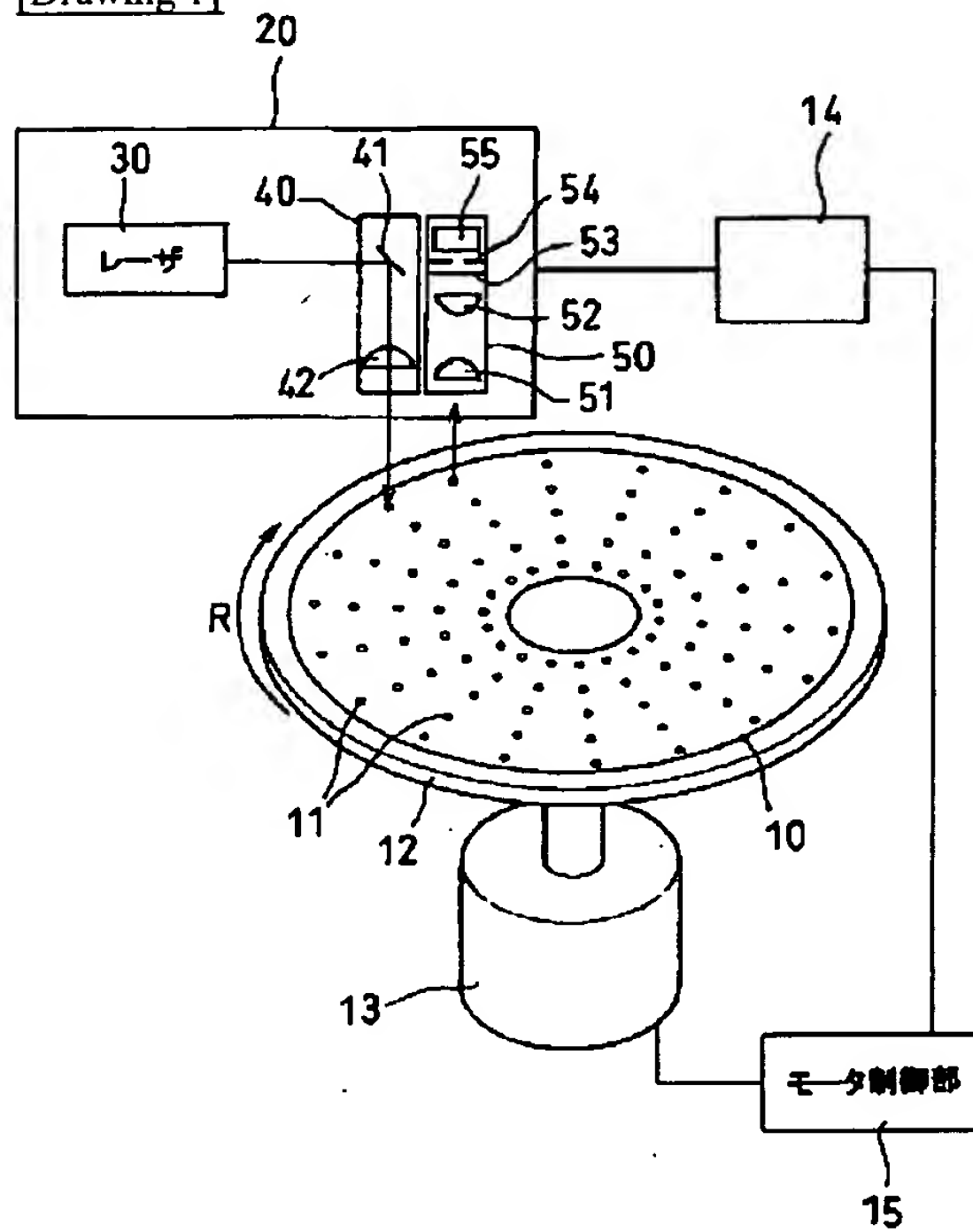
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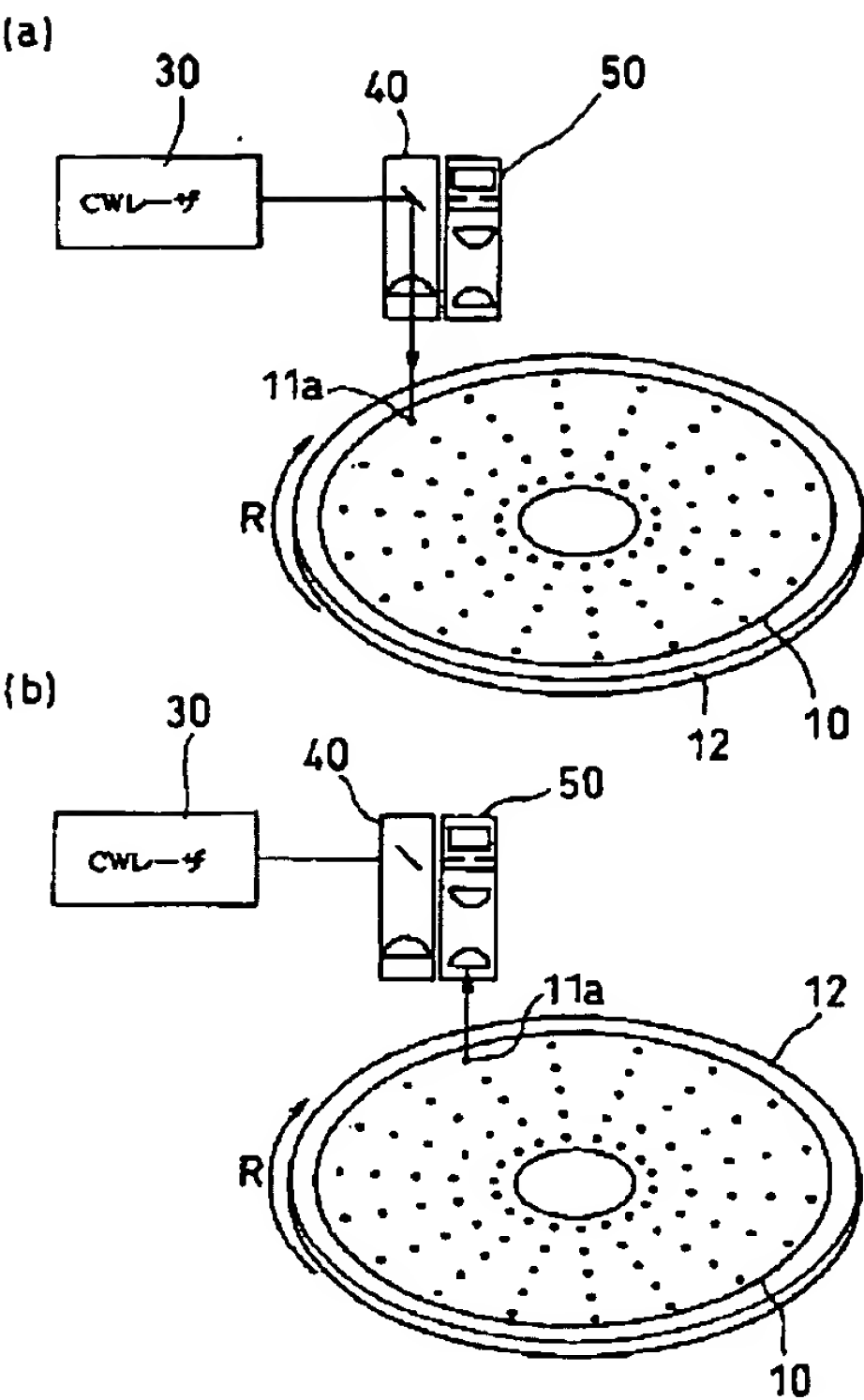
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DRAWINGS

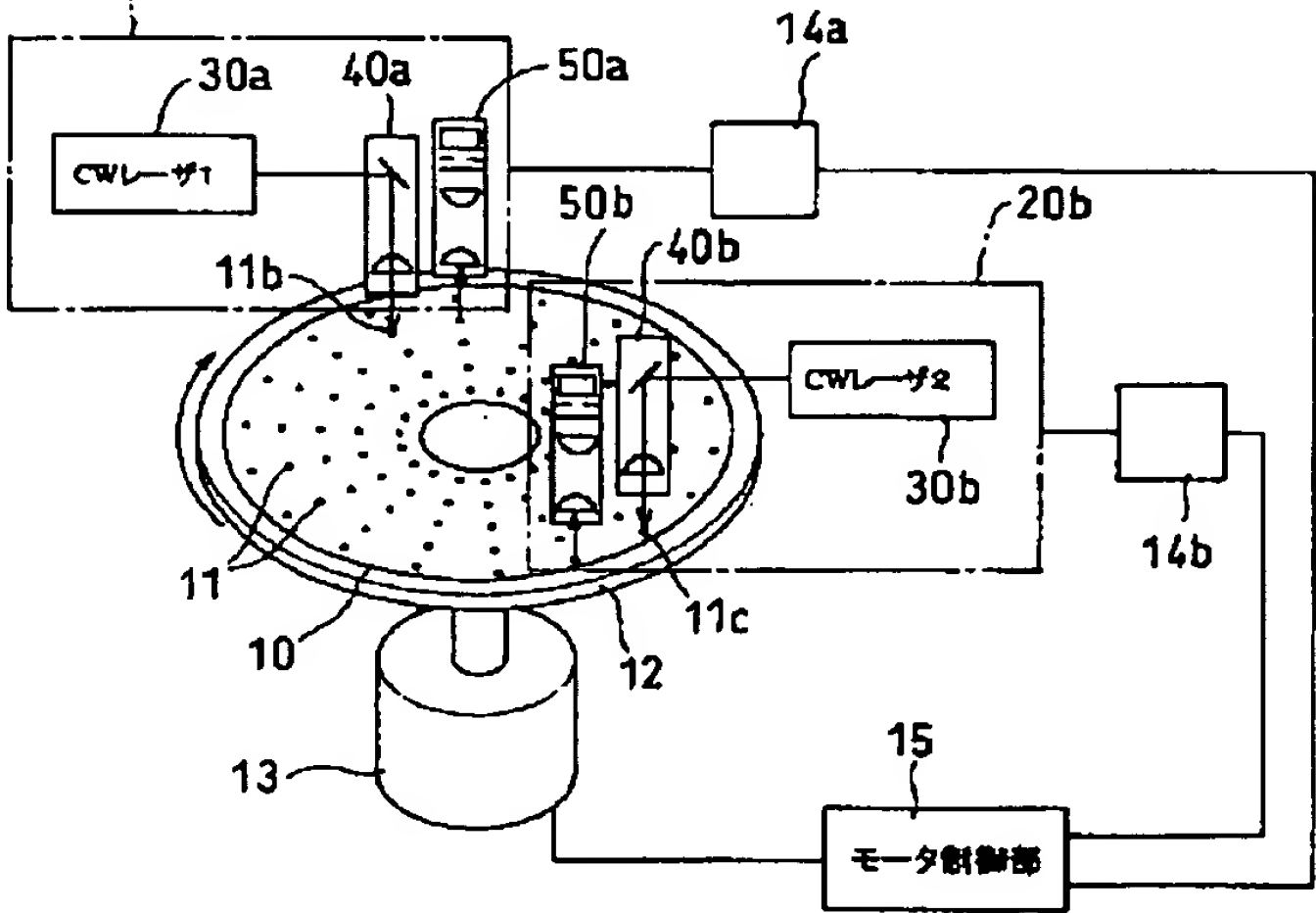
[Drawing 1]



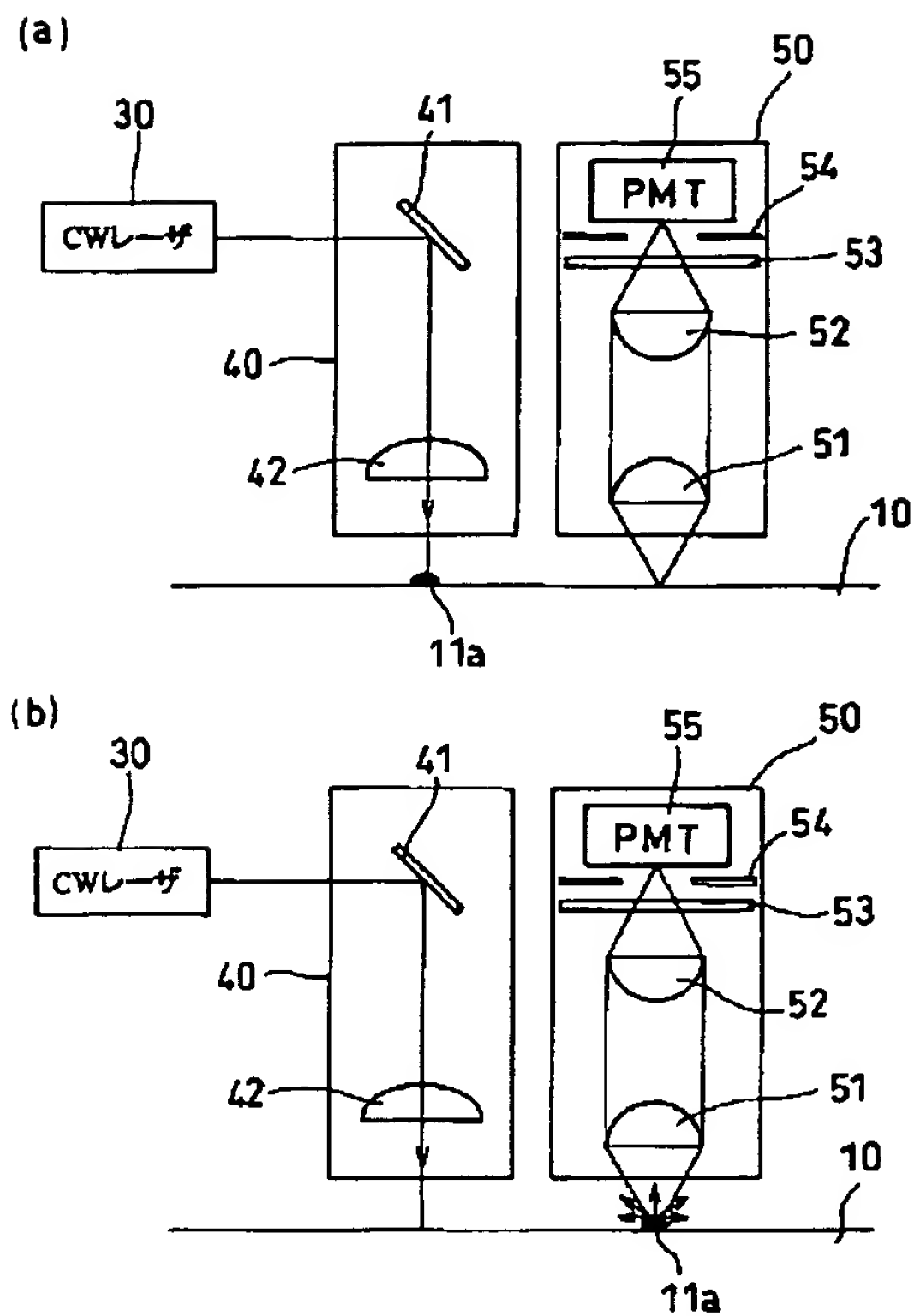
[Drawing 2]



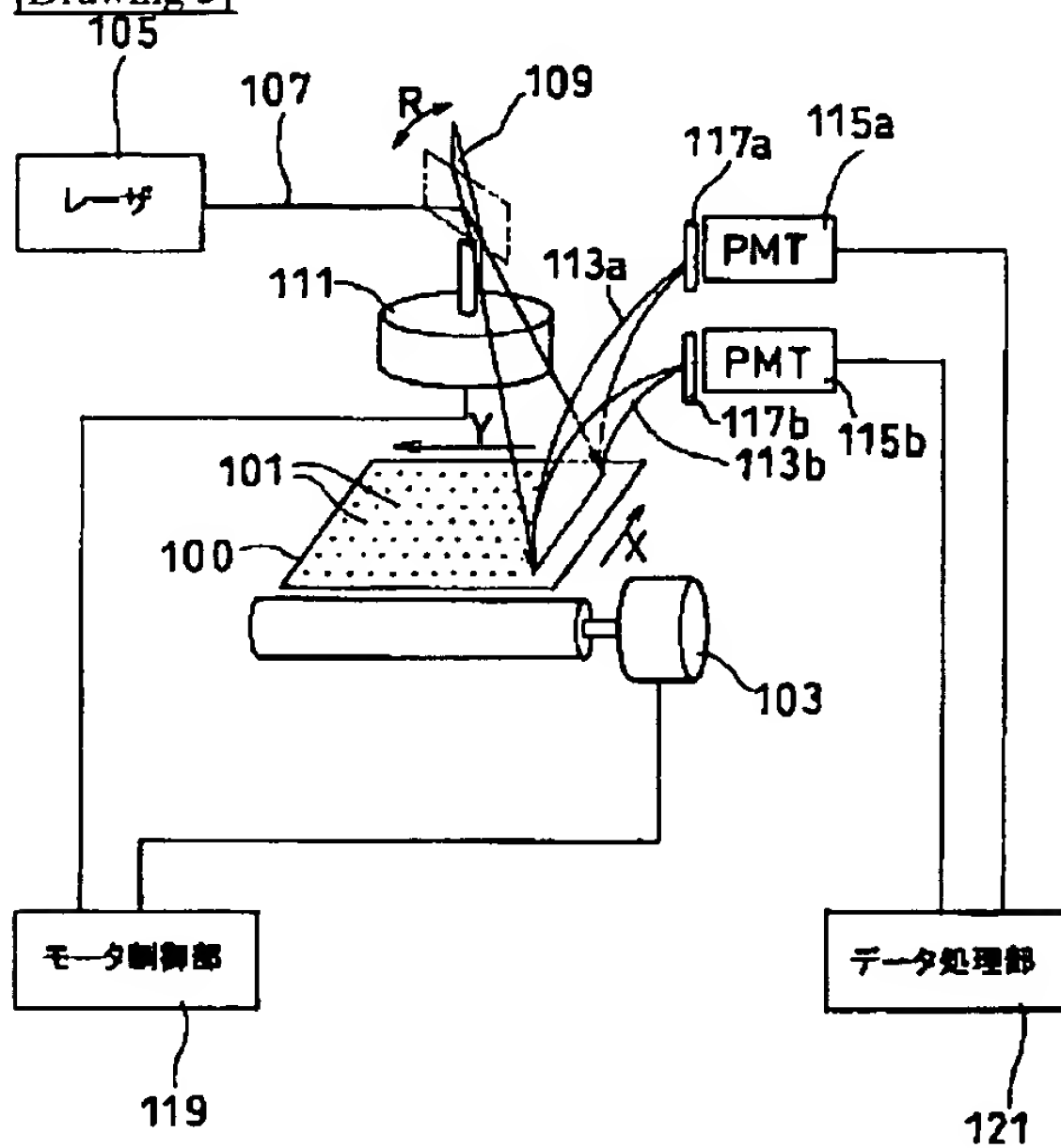
[Drawing 4]
20a



[Drawing 3]



[Drawing 5]



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TECHNICAL FIELD

[The technical field to which invention belongs] Especially this invention relates to the fluorescence photometry method for reading of the biochip arranged by the plane with suitable samples by which the indicator was carried out with the fluorescent substance, such as DNA and protein, and fluorescence photometry equipment about the fluorescence photometry method and fluorescence photometry equipment.

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EFFECT OF THE INVENTION

[Effect of the Invention] According to this invention, the fluorescence from the fluorescent substance contained in a sample spot can be measured to high sensitivity, without being influenced of excitation light.

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PRIOR ART

[Description of the Prior Art] In the field of molecular biology or biochemistry, search, a sick diagnosis, etc. of a useful gene are performed using the hybridization reaction which makes a nucleic acid and protein with a known array, and the target molecule in a sample hybridize. In that case, in order to process a lot of samples for a short time, the biochip which arranged many sample spots is used for a front face. A probe different, respectively is being fixed to each sample spot on a biochip, this biochip is put in into a reaction container with Sample DNA, and hybridization with the sample DNA which carried out fluorescent labeling to the probe combined with each sample spot of a biochip in it is performed. Then, by irradiating excitation light at a biochip and measuring the fluorescence intensity emitted from each sample spot with fluorescence photometry equipment, the amount of combination of each probe and Sample DNA is known, and it is changed into required information.

[0003] Drawing 5 is the schematic diagram of the conventional fluorescence photometry equipment used for reading of a biochip. This fluorescence photometry equipment performs excitation light irradiation with a light-spot scanning mode to each sample spot on a biochip, and is made to perform incorporation of the fluorescence emitted from each sample spot by the optical fiber bundle.

[0004] The sample spot 101 which contains DNA by which the indicator was carried out with the fluorescent substance, protein, etc. in the front face of the biochip 100 which consists of a slide glass etc. is arranged in the shape of a grid at intervals of about 100 micrometers in the direction of Y as a minute spot with a diameter of 50 micrometers. The parallel displacement of the biochip 100 is carried out in the direction shown by Arrow Y by the chip delivery motor 103. It is reflected by the rotation mirror 109, and the laser beam 107 generated from laser 105 serves as the light spot on the surface of a biochip, and is drawn. The rotation mirror 109 is rotated in the direction of arrow R by the motor 111. Therefore, a laser beam 107 carries out rectilinear scanning of the front face of a biochip 100 in the direction of arrow X. Thus, the chip delivery motor 103 and the motor 111 were controlled by the motor control section 119, the laser beam was scanned in the direction of X, and optical irradiation of the whole sample side of a biochip 100 was carried out by moving a biochip in the direction of Y serially.

[0005] The fluorescence generated from each sample spot of a biochip is led to the photomultiplier tubes (photomultiplier tube;PMT) 115a and 115b by optical fiber bundles 113a and 113b. The incidence side of optical fiber bundles 113a and 113b is arranged in the shape of a line, and is turned to the laser beam scanning line of biochip 1 front face. Moreover, the other end of optical fiber bundles 113a and 113b is bundled, and is arranged towards PMT(s) 115a and 115b. Between optical fiber bundles 113a and 113b, PMT115a, and 115b, light filters 117a and 117b are arranged, and only the target fluorescence wavelength is read by PMT(s) 115a and 115b. Data processing of the output of PMT(s) 115a and 115b is sent and carried out to the data-processing section 121. Thus, by installing two or more light-receiving systems equipped with the light filter with which transmitted wave length regions differ, multicolor read was made possible.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] The excitation laser beam to which the above-mentioned conventional fluorescence photometry equipment was reflected or scattered about at the light-receiving system not only in respect of the fluorescence from a sample but in respect of the sample was contained. Since a sample is a minute amount, several figures of the quantity of light of the excitation light which carries out incidence to a light-receiving system are also larger than the quantity of light of fluorescence. Therefore, in order to remove excitation light, as a light filter, what has a narrow transmitted wave length band needed to be used, and the fluorescence which should be detected might also be omitted with the light filter. Moreover, the optical fiber bundle used for incorporation of fluorescence needed the optical fiber of a number equal to the number of a sample at least for the direction of light scanning, and when becoming disadvantageous in respect of cost, optical-axis doubling of an optical fiber had taken a precise mechanism and precise adjustment to it.

[0007] Moreover, since an optical fiber has little fluorescence which a light-receiving angle can incorporate from a sample small, it has the inclination for a S/N ratio to become low. although the attempt which is made to decrease the intensity of an excitation laser beam and receives only the fluorescence of a sample immediately after using a pulse laser and an acoustooptic modulator (acoustooptic modulator; AOM), and exciting a sample in order to raise a S/N ratio also occurs -- a pulse laser -- expensive -- AOM -- the intensity of a laser beam -- about -- it was not what it is made to only decrease to 1/1000, and can prevent mixing of an excitation laser beam completely

[0008] this invention solves the trouble in the conventional technology mentioned above, and aims at offering the fluorescence photometry method and fluorescence photometry equipment which can acquire a high detection precision easily with easy composition, using comparatively cheap laser, such as a continuous wave laser (continuous-wave laser; CW laser), as the excitation light source.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The schematic diagram showing an example of the fluorescence photometry equipment by this invention.

[Drawing 2] The ** type view explaining the physical relationship of excitation light irradiation optical system and fluorescence detection optical system.

[Drawing 3] The ** type view explaining the principle of the fluorescence detection by this invention.

[Drawing 4] The schematic diagram explaining other examples of the fluorescence photometry equipment by this invention.

[Drawing 5] The schematic diagram of conventional fluorescence photometry equipment.

[Description of Notations]

10 -- A biochip, 11, 11a, 11b, 11c -- Sample spot, 12 [-- Head delivery motor,] -- A sample base, 13 -- A rotary motor, 14, 14a, 14b 15 [-- Excitation light source,] -- The motor control section, 20, 20a, 20b -- An optical head, 30, 30a, 30b 40, 40a, 40b [-- Objective lens,] -- Excitation light irradiation optical system, 41 -- A mirror, 42 50, 50a, 50b [-- Condenser lens,] -- Fluorescence detection optical system, 51 -- Light-receiving lenses 51 and 52 53 [-- A light sensitive cell, 100 / -- Biochip,] -- A fluorescence selection filter, 54 -- A slit, 55 101 [-- Laser,] -- A sample spot, 103 -- A chip delivery motor, 105 107 [-- A motor, 113a, 113b / -- An optical fiber bundle, 115a 115b / -- The photomultiplier tube, 117a 117b / -- A light filter, 119 / -- The motor control section, 121 / -- Data-processing section] -- A laser beam, 109 -- A rotation mirror, 111

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technical field to which invention belongs] Especially this invention relates to the fluorescence photometry method for reading of the biochip arranged by the plane with suitable samples by which the indicator was carried out with the fluorescent substance, such as DNA and protein, and fluorescence photometry equipment about the fluorescence photometry method and fluorescence photometry equipment.

[0002]

[Description of the Prior Art] In the field of molecular biology or biochemistry, search, a sick diagnosis, etc. of a useful gene are performed using the hybridization reaction which makes a nucleic acid and protein with a known array, and the target molecule in a sample hybridize. In that case, in order to process a lot of samples for a short time, the biochip which arranged many sample spots is used for a front face. A probe different, respectively is being fixed to each sample spot on a biochip, this biochip is put in into a reaction container with Sample DNA, and hybridization with the sample DNA which carried out fluorescent labeling to the probe combined with each sample spot of a biochip in it is performed. Then, by irradiating excitation light at a biochip and measuring the fluorescence intensity emitted from each sample spot with fluorescence photometry equipment, the amount of combination of each probe and Sample DNA is known, and it is changed into required information.

[0003] Drawing 5 is the schematic diagram of the conventional fluorescence photometry equipment used for reading of a biochip. This fluorescence photometry equipment performs excitation light irradiation with a light-spot scanning mode to each sample spot on a biochip, and is made to perform incorporation of the fluorescence emitted from each sample spot by the optical fiber bundle.

[0004] The sample spot 101 which contains DNA by which the indicator was carried out with the fluorescent substance, protein, etc. in the front face of the biochip 100 which consists of a slide glass etc. is arranged in the shape of a grid at intervals of about 100 micrometers in the direction of Y as a minute spot with a diameter of 50 micrometers. The parallel displacement of the biochip 100 is carried out in the direction shown by Arrow Y by the chip delivery motor 103. It is reflected by the rotation mirror 109, and the laser beam 107 generated from laser 105 serves as the light spot on the surface of a biochip, and is drawn. The rotation mirror 109 is rotated in the direction of arrow R by the motor 111. Therefore, a laser beam 107 carries out rectilinear scanning of the front face of a biochip 100 in the direction of arrow X. Thus, the chip delivery motor 103 and the motor 111 were controlled by the motor control section 119, the laser beam was scanned in the direction of X, and optical irradiation of the whole sample side of a biochip 100 was carried out by moving a biochip in the direction of Y serially.

[0005] The fluorescence generated from each sample spot of a biochip is led to the photomultiplier tubes (photomultiplier tube;PMT) 115a and 115b by optical fiber bundles 113a and 113b. The incidence side of optical fiber bundles 113a and 113b is arranged in the shape of a line, and is turned to the laser beam scanning line of biochip 1 front face. Moreover, the other end of optical fiber bundles 113a and 113b is bundled, and is arranged towards PMT(s) 115a and 115b. Between optical fiber bundles 113a and 113b, PMT115a, and 115b, light filters 117a and 117b are arranged, and only the target fluorescence wavelength is read by PMT(s) 115a and 115b. Data processing of the output of PMT(s) 115a and 115b is sent and carried out to the data-processing section 121. Thus, by installing two or more light-receiving systems equipped with the light filter with which transmitted wave length regions differ, multicolor read was made possible.

[0006]

[Problem(s) to be Solved by the Invention] The excitation laser beam to which the above-mentioned conventional fluorescence photometry equipment was reflected or scattered about at the light-receiving system not only in respect of the fluorescence from a sample but in respect of the sample was contained. Since a sample is a minute amount, several figures of the quantity of light of the excitation light which carries out incidence to a light-receiving system are also larger than the quantity of light of fluorescence. Therefore, in order to remove excitation light, as a light filter, what has a narrow transmitted wave length band needed to be used, and the fluorescence which should be detected might also be omitted with the light filter. Moreover, the optical fiber bundle used for the incorporation of fluorescence needed the optical fiber of a number equal to the number of a sample at least for the direction of light scanning, and when becoming disadvantageous in respect of cost, optical-axis doubling of an optical fiber had taken a precise mechanism and precise adjustment to it.

[0007] Moreover, since an optical fiber has little fluorescence which a light-receiving angle can incorporate from a sample small, it has the inclination for a S/N ratio to become low. although the attempt which is made to decrease the intensity of an excitation laser beam and receives only the fluorescence of a sample immediately after using a pulse laser and an acoustooptic modulator

(acoustooptic modulator; AOM), and exciting a sample in order to raise a S/N ratio also occurs -- a pulse laser -- expensive -- AOM -- the intensity of a laser beam -- about -- it was not what it is made to only decrease to 1/1000, and can prevent mixing of an excitation laser beam completely

[0008] this invention solves the trouble in the conventional technology mentioned above, and aims at offering the fluorescence photometry method and fluorescence photometry equipment which can acquire a high detection precision easily with easy composition, using comparatively cheap laser, such as a continuous wave laser (continuous-wave laser; CW laser), as the excitation light source.

[0009]

[Means for Solving the Problem] In this invention, the excitation light irradiation section and a fluorescence detecting element are detached spatially, and are set up, the sample by which excitation light was irradiated in the excitation light irradiation section is moved to a fluorescence detecting element from the excitation light irradiation section, a S/N ratio is improved by performing fluorescence detection in the state where there is no influence of excitation light at a fluorescence detecting element, and the aforementioned purpose is attained.

[0010] That is, the fluorescence photometry method by this invention is characterized by including the step which irradiates the excitation light from the excitation light source at a sample, the step which moves the sample which has irradiated excitation light onto the optical axis of a fluorescence detection means, and the step which detects the fluorescence which carried out incidence to the fluorescence detection means. Let a sample be a sample containing the biopolymer by which the indicator was carried out with the fluorescent substance.

[0011] By this method, the sample which has irradiated excitation light is moved within a time [short] as compared with the life of the fluorescence generated from a sample onto the optical axis of the fluorescence detection means turned to the excitation light irradiation position and a different position, and fluorescence detection is performed. Therefore, since the excitation light which set in the excitation light irradiation position, and was reflected or scattered about from the sample does not carry out incidence to a fluorescence detection means, only fluorescence is detectable by high sensitivity.

[0012] The excitation light irradiation means for the fluorescence photometry equipment by this invention irradiating the excitation light from the excitation light source and the excitation light source at a sample, The fluorescence detection means for detecting the fluorescence which has the optical axis of an excitation light irradiation means, and the optical axis which does not cross on a sample, and was emitted by excitation light irradiation from the sample, It is characterized by having a sample move means for moving the sample which has irradiated excitation light by the excitation light irradiation means onto the optical axis of a fluorescence detection means.

[0013] As for this fluorescence photometry equipment, the optical axis of an excitation light irradiation means and the optical axis of a fluorescence detection means do not cross on a sample. Therefore, although a fluorescence photometry cannot be performed to the sample located under the excitation light irradiation means, as compared with the life time of fluorescence, a sample is moved to within a time [short] under the fluorescence detection means, and fluorescence detection is performed. Thus, in order that the excitation light which set in the excitation light irradiation position, and was reflected or scattered about from the sample by setting a fluorescence irradiation position and a fluorescence detection position as a spatially different position may not carry out incidence to a fluorescence detection means, it becomes possible to detect only fluorescence by high sensitivity.

[0014] As for a fluorescence detection means, it is desirable to have the confocal point optical system with which photodetection meanses, such as PMT, and the photodetection side and sample side of a photodetection means became conjugate. Moreover, a sample move means can be made into a means to rotate a sample, and in order to detect the fluorescence of different wavelength, it can be equipped with two or more sets of groups of the aforementioned excitation light source, an excitation light irradiation means, and a fluorescence detection means.

[0015] the fluorescent substance used for this invention has the desirable long thing of the life time of fluorescence, and 4 which is the fluorescent substance of Eu (europium) complex, 4'-bis(one -- " -- one -- " -- one -- " -- two -- " -- two -- " -- three -- " -- three -- " -- heptafluoro-4" and 6"-hexanedion-6"-yl) chlorosulfo-o-terphenyl (abbreviated name : BHHCT), a rhodamine, FITC, Cy3, Cy5, etc. are suitable for it For example, BHHCT is the fluorescent substance which is excited on the wavelength of 340nm and gives off fluorescence with a wavelength of 615nm, and its half-life of fluorescence is very long compared with the half-life (dozens of ns) of 100-200microsec and the conventional fluorescent substance. If this property is used, after moving the sample which carried out excitation light irradiation, even if it carries out fluorescence detection, many amounts of fluorescence can be obtained, and a S/N ratio can be improved by leaps and bounds.

[0016]

[Embodiments of the Invention] Hereafter, the gestalt of operation of this invention is explained with reference to a drawing.

Drawing 1 is the schematic diagram showing an example of the fluorescence photometry equipment by this invention. This equipment is suitable fluorescence photometry equipment for reading of a circular biochip. The shape of a concentric circle and the circular biochip 10 by which many sample spots 11 are arranged spirally are held on the sample base 12, and the rotation drive of the sample base 12 is carried out in the direction of Arrow R by the rotary motor 13. Above the biochip 10, the optical head 20 equipped with the excitation light source 30, the excitation light irradiation optical system 40, and the fluorescence detection optical system 50 is arranged. For example, on the sample spot 11 with a diameter of 50 micrometers which is a minute spot, biopolymers by which the indicator was carried out with the fluorescent substance, such as DNA and protein, have hybridized with probe DNA etc.

[0017] As the excitation light source 30, a CW laser is used, for example. Incidence is carried out to the excitation light

irradiation optical system 40, and it is reflected by the mirror 41, it converges with an objective lens 42, and the laser beam generated from the excitation light source 30 is irradiated by the sample spot on a biochip 10. The fluorescence detection optical system 50 is equipped with the photomultiplier tube as the light-receiving lens 51, a condenser lens 52, the fluorescence selection filter 53, a slit 54, and a light sensitive cell 55, and detects the fluorescence generated from the sample spot.

[0018] The optical head 20 is moved and positioned by radial [of a biochip 10] by the head delivery motor 14. The optical head 20 is continuously moved so that the head delivery motor 14 may pursue the sample spot 11 with the spiral head delivery motor 14, when the optical head 20 is positioned [as opposed to / each concentric circle of a sample spot / when the sample spot is arranged in the shape of 11 concentric circles on the biochip 10] in step and the sample spot 11 is spirally arranged on the biochip 10. The motor control section 15 controls a rotary motor 13 and the head delivery motor 14, and it carries out adjustable control of the rotational speed of a rotary motor 13 according to the radius position of the optical head 20 on a biochip 10 so that the traverse speed of the sample spot 11 in the lower part position of the optical head 20 may become always fixed.

[0019] Drawing 2 is a ** type view explaining the physical relationship of excitation light irradiation optical system and fluorescence detection optical system. The excitation light generated from the excitation light source (CW laser) 30 is irradiated through the excitation light irradiation optical system 40 by one sample spot 11a on the revolving biochip 10, and drawing 2 (a) shows the state where the fluorescent substance in sample spot 11a is excited. Then, as shown in drawing 2 (b), sample spot 11a by which excitation light was irradiated moves with rotation of a biochip 10, and comes just under the fluorescence detection optical system 50. The fluorescence generated from sample spot 11a carries out incidence to the fluorescence detection optical system 50, and is detected in this position.

[0020] Drawing 3 is a ** type view explaining the principle of the fluorescence detection by this invention. Drawing 3 (a) shows the state where the excitation light from CW laser 30 is irradiated by sample spot 11a of biochip top 10 which is moving. If the indicator fluorescent substance is contained in sample spot 11a, fluorescence will be emitted from sample spot 11a which has irradiated excitation light. The sample spot which passed through the lower part position of the excitation light irradiation optical system 40, and received excitation light irradiation arrives at the lower part position of the fluorescence detection optical system 50, as shown in drawing 3 (b) after fixed time progress after excitation light irradiation. The light-receiving lens 51 and condenser lens 52 of the fluorescence detection optical system 50 constitute confocal point optical system. Although the fluorescence emitted from sample spot 11a spreads in an omnidirection, it converges with a condenser lens 52, and a noise is removed by passing along a light filter 53 and a slit 54, incidence of the fluorescence which carried out incidence to light-receiving NZU 51 which constitutes confocal point optical system is carried out to a light sensitive cell (PMT) 55, and it is detected. In this way, it becomes possible to perform a fluorescent light measurement, without being influenced of excitation light. In addition, although it has illustrated to drawing 3 so that the optical axis of the excitation light irradiation optical system 40 and the optical axis of the fluorescence detection optical system 50 may become parallel, the optical axis of two optical system 40 and 50 does not necessarily need to be parallel.

[0021] Drawing 4 is a schematic diagram explaining other examples of the fluorescence photometry equipment by this invention. This fluorescence photometry equipment is equipped with two optical heads 20a and 20b, and discriminates and detects the fluorescence emitted from two kinds of indicator fluorescent substances contained in a sample spot. Optical head 20a is equipped with excitation light source (CW laser) 30a, excitation light irradiation optical-system 40a, and fluorescence detection optical-system 50a, and optical head 20b is equipped with excitation light source (CW laser) 30b, excitation light irradiation optical-system 40b, and fluorescence detection optical-system 50b. Since the detailed composition of the excitation light irradiation optical system 40a and 40b and the detailed composition of the fluorescence detection optical system 50a and 50b are the same as the detailed composition of the excitation light irradiation optical system 40 shown in drawing 1, and the fluorescence detection optical system 50, explanation is omitted here.

[0022] However, excitation wavelength is chosen so that the excitation light sources 30a and 30b can excite a fluorescent substance different, respectively efficiently, and the fluorescence detection optical system 50a and 50b is equipped with the light filter with which the transmitted wave length band was chosen so that the fluorescence from a fluorescent substance different, respectively could be detected efficiently. In this way, one optical head 20a detects alternatively Cy3 which irradiates the excitation light from excitation light source 30a by excitation light irradiation optical-system 40a at sample spot 11b, measures the strength of the light, without excitation light interfering with the fluorescence generated from sample spot 11b by fluorescence detection optical-system 50a after fixed time, for example, is contained in sample spot 11b. Moreover, optical head 20b of another side detects alternatively Cy5 which irradiates the excitation light from excitation light source 30b by excitation light irradiation optical-system 40b at sample spot 11c, measures the strength of the light, without excitation light interfering with the fluorescence generated from sample spot 11c by fluorescence detection optical-system 50b after fixed time, for example, is contained in sample spot 11c.

[0023] It is moved to radial [of a biochip 10] by the head delivery motors 14a and 14b, and two optical heads 20a and 20b are positioned by them in the almost same radius position. When the sample spot is arranged in the shape of 11 concentric circles on the biochip 10 The head delivery motors 14a and 14b position the optical heads 20a and 20b in step to each concentric circle of a sample spot. When the sample spot 11 is spirally arranged on the biochip 10, the head delivery motors 14a and 14b move the optical heads 20a and 20b continuously so that the spiral sample spot 11 may be pursued. the motor control section 15 controls a rotary motor 13 and the head delivery motors 14a and 14b, and it carries out adjustable control of the rotational speed of a rotary motor 13 according to the radius position of the optical heads 20 and 20b on a biochip 10 so that the traverse speed of the sample spot 11 in the lower part position of the optical heads 20a and 20b may always serve as about 1 law

[0024]

[Effect of the Invention] According to this invention, the fluorescence from the fluorescent substance contained in a sample spot can be measured to high sensitivity, without being influenced of excitation light.

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CLAIMS

[Claim(s)]

[Claim 1] The fluorescence photometry method characterized by including the step which irradiates the excitation light from the excitation light source at a sample, the step which moves the sample which has irradiated excitation light onto the optical axis of a fluorescence detection means, and the step which detects the fluorescence which carried out incidence to the aforementioned fluorescence detection means.

[Claim 2] It is the fluorescence photometry method characterized by including the biopolymer to which the indicator of the sample was carried out with the fluorescent substance in the fluorescence photometry method according to claim 1.

[Claim 3] Fluorescence photometry equipment characterized by providing the following. Excitation light source. The excitation light irradiation means for irradiating the excitation light from the aforementioned excitation light source at a sample. The fluorescence detection means for detecting the fluorescence which has the optical axis of the aforementioned excitation light irradiation means, and the optical axis which does not cross on a sample, and was emitted by excitation light irradiation from the sample. The sample move means for moving the sample which has irradiated excitation light by the aforementioned excitation light irradiation means onto the optical axis of the aforementioned fluorescence detection means.

[Claim 4] It is fluorescence photometry equipment which is a means by which the aforementioned sample move means rotates a sample in fluorescence photometry equipment according to claim 3, and is characterized by having two or more sets of groups of the aforementioned excitation light source, an excitation light irradiation means, and a fluorescence detection means in order to detect the fluorescence of different wavelength.

[Translation done.]